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**ARTICLE**

# Fragmentation and patch size shape genetic structure of brook trout populations

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**Abstract:** We tested the relative influence of habitat patch size and connectivity on genetic structure and effective population size in eight brook trout (*Salvelinus fontinalis*) habitat patches in a watershed in Virginia, USA. Variation at eight microsatellite loci in 2229 young-of-the-year brook trout for two successive cohorts (2010 and 2011) was examined. Genetic differentiation across all populations was pronounced. Overall $F'_{ST}$ was 0.397 (95% CI: 0.322–0.525) and overall $F_{ST}$ was 0.124 (95% CI: 0.096–0.159). Above-barrier patch size had a strong positive relationship with genetic diversity, $\hat{N}_b$, and genetic differentiation. Our analysis is consistent with greater extinction risk in smaller above-barrier patches. Larger above-barrier patches contained greater genetic diversity but reduced $\hat{N}_b$ relative to adjacent below-barrier patches. The primary effect of barriers may be to reduce available above-barrier spawning habitat, even for larger above-barrier patches. Below-barrier patches also showed evidence of reduced genetic diversity and lack of connectivity. Genetic monitoring focused at gaining a broader understanding of the relationships here will be necessary to fully evaluate local extinction risks.

**Résumé :** Nous avons testé l'influence relative de la taille et de la connectivité des parcelles d'habitat sur la structure génétique et la taille effective de la population dans huit parcelles d'habitat de l'omble de fontaine (*Salvelinus fontinalis*) dans un bassin versant en Virginie (États-Unis). Les variations en huit loci de microsatellites chez 2229 jeunes de l'année pour deux cohortes successives (2010 et 2011) d'ombles de fontaine ont été examinées. La différentiation génétique entre les différentes populations était marquée. Le $F'_{ST}$ global était de 0,397 (IC à 95 % : 0,322–0,525) et le $F_{ST}$ global, de 0,124 (IC à 95 % : 0,096–0,159). La taille des parcelles en amont de barrières présentait une forte relation positive avec la diversité génétique,, et la différentiation génétique. Notre analyse concorde avec un risque de disparition accru dans les parcelles de tailles plus petites en amont de barrières. Les parcelles de plus grandes tailles en amont de barrières présentaient une plus grande diversité génétique, mais des $\hat{N}_b$ plus faibles comparativement aux parcelles attenantes en aval de barrières. Le principal effet des barrières pourrait être une moins grande disponibilité d'habitats de frai en amont de barrières et ce, même pour les plus grandes parcelles en amont de barrières. Les parcelles en aval de barrières présentaient également des signes de diversité génétique réduite et d'absence de connectivité. Une surveillance génétique visant à établir une compréhension plus large de ces relations sera nécessaire à une évaluation exhaustive des risques de disparition locale. [Traduit par la Rédaction]

## Introduction

Landscape changes (deforestation, dams, road systems, impassable culverts, invasive species) have greatly reduced available habitat and connectivity among populations of headwater stream fishes (Dunham et al. 1997; Morita and Yamamoto 2002; Letcher et al. 2007). The size of discrete headwater stream habitat areas or "patches" appears to be closely related to population persistence in headwater salmonids (Isaak et al. 2007; Dunham et al. 2008). A habitat patch for a headwater salmonid is generally defined as a continuous network of thermally suitable habitat (Isaak et al. 2010). Larger patches tend to support larger populations, which may alleviate the effects of environmental and demographic stochasticity (Lande 1993). Larger patches are also likely to have enough habitat heterogeneity to meet the diverse habitat requirements of salmonids (Harig and Fausch 2002). Fragmentation in headwater streams, either owing to natural (e.g., waterfalls) or anthropogenic (e.g., dams, culverts, irrigation diversions) sources, should have a strong effect on the relationship between patch

size and population persistence (Neville et al. 2006). Upon isolation, relatively small above-barrier patches have been shown to be more susceptible to extirpation in three salmonid species (Harig and Fausch 2002; Morita and Yamamoto 2002; Koizumi 2011).

Patch size and connectivity should also interact in their effects on genetic variation (Neville et al. 2006), but the strength of the relationship should vary among sites and species. There is substantial evidence for loss of genetic diversity above natural and anthropogenic barriers for a variety of salmonids (Angers et al. 1999; Bouza et al. 1999; Carlsson and Nilsson 1999; Costello et al. 2003; Taylor et al. 2003, Wofford et al. 2005; Neville et al. 2006; Guy et al. 2008; Kitanishi et al. 2012). There is also evidence for a strong positive relationship among above-barrier patch size, genetic diversity, and effective population size (Whiteley et al. 2010; Peacock and Dochtermann 2012). However, this relationship has been weak in other studies (Castric et al. 2001; Yamamoto et al. 2004; Whiteley et al. 2006). Additional work is needed to understand the effects of patch size and connectivity on genetic diver-

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**Table 1.** Sample information including site names, abbreviations, sample years, sample sizes of young of the year ($N_{YOY}$), patch area (ha), and stream length (km).

| Site name | Site code | Sample year | $N_{YOY}$ | Patch area (ha) | Stream length (km) | Patch size | Dam age | YOY $\hat{N}_c$ | Adult $\hat{N}_c$ |
|---|---|---|---|---|---|---|---|---|---|
| Dry River-a | DV-a | 2010 | 379 | 3807 | 27.4 | 104.3 | 1970 | 1285 (843–2077) | 1982 (1726–2202) |
| Dry River-a | DV-a | 2011 | 510 | | | | | 1009 (795–1275) | 616 (529–719) |
| Dry River-b | DV-b | 2010 | 99 | 10 880 | 40.0 | 435.2 | — | — | — |
| Dry River-b | DV-b | 2011 | 67 | | | | | | |
| Dry Run-a | DN-a | 2010 | 46 | 1217 | 8.1 | 9.9 | 1968 | 117 (86–367) | 83 (78–156) |
| Dry Run-a | DN-a | 2011 | 27 | | | | | 22 (21–23) | 43 (40–45) |
| Briery Branch-a | BB-a | 2010 | 72 | 2438 | 6.1 | 14.9 | 1966 | 236 (139–457) | 366 (296–576) |
| Briery Branch-a | BB-a | 2011 | 90 | | | | | 139 (91–215) | 129 (104–175) |
| Briery Branch-b | BB-b | 2011 | 98 | 3925 | 19.1 | 75.0 | — | — | — |
| Little River-a | LR-a | 2010 | 299 | 4121 | 12.7 | 52.3 | 1965 | 463 (347–633) | 728 (637–873) |
| Little River-a | LR-a | 2011 | 377 | | | | | 677 (519–882) | 323 (236–438) |
| Little River-b | LR-b | 2011 | 57 | 2450 | 10.8 | 26.5 | — | | |
| Skidmore Fork-a | SF-a | 2010 | 58 | 993 | 5.1 | 5.1 | 1962 | 47 (42–130) | 268 (231–346) |
| Skidmore Fork-a | SF-a | 2011 | 50 | | | | | 70 (50–117) | 90 (73–130) |

**Note:** Patch size was obtained by multiplication of patch area × stream length × $10^{-3}$. Dam age is the year of dam construction and is only provided for above-barrier patches. Patch metrics and dam age are listed only once for each patch. Mark–recapture-based estimates of population census size ($\hat{N}_c$) are presented separately for YOY and adults (over-yearlings) and were collected for the above-dam patches only.

sity, effective population size, and population persistence in headwater fish populations, particularly as available habitat and population connectivity are likely to continue to decline with future climate change effects because of alterations in stream flow and temperature suitability (Hari et al. 2006; Hudy et al. 2008; Isaak et al. 2010; Wenger et al. 2011).

The brook trout (*Salvelinus fontinalis*) is a sentinel cold-water salmonid with a native range in headwater streams of eastern North America from northern Georgia to the south through most of eastern Canada to the north (Page and Burr 1991). Factors that influence maintenance of genetic diversity and promotion of genetic differentiation have been explored in northern portions of this species' native range (Castric et al. 2001; Castric and Bernatchez 2003, 2004; Fraser and Bernatchez 2005; Fraser et al. 2005). An analysis of strictly freshwater populations under relatively pristine conditions in northern Maine, USA, found that elevation but not patch size was related to within-population genetic diversity (Castric et al. 2001). More southern brook trout populations in the USA have been more anthropogenically influenced than northern populations (Hudy et al. 2008). Southern populations tend to occur in small habitat patches that we define for this species as a group of occupied contiguous catchments (Hudy et al. 2008). For example, the median size of over 2800 habitat patches in the southeastern USA is 855 ha (M. Hudy, unpublished results). Many dams isolate populations in upstream patches (Hudy et al. 2008; Bain and Wine 2010). Even patches below anthropogenic (dams) or natural (waterfall) barriers may show effects of isolation because of warm water habitat (Meisner 1990) or downstream invasive species (Moore et al. 1983, 1986; Strange and Habera 1998). Brook trout habitat patch size may be a readily obtainable metric with high conservation utility if it is closely related to maintenance of genetic diversity and population persistence in the most imperiled portions of the species range. To establish the utility of the patch concept for brook trout, it is necessary to explore the linkages among patch size, connectivity, genetic diversity, and population persistence.

Here, we examine the relative influence of patch size and connectivity on genetic structure and effective population size of a series of brook trout populations in the Appalachian Mountains, Virginia, USA. We had two primary objectives: (*i*) to characterize genetic variation within and genetic divergence among eight predefined above- or below-dam habitat patches and (*ii*) to test the relationship between patch size and within-patch genetic diver-

sity or effective population size for patches either above or below dams.

## Methods

### Brook trout sampling

We defined a patch for brook trout as a group of occupied contiguous catchment polygons from the US Geological Survey (USGS) National Hydrology Dataset (NHD) Plus catchment GIS layer (seventh level, 14-digit hydrolic unit codes). We began a patch in the catchment polygon where a brook trout population had been documented based on previous occurrence data and then expanded that patch (dissolving catchment polygon boundaries) to include all catchment polygons upstream until a barrier to fish passage, such as a dam or lake, was encountered or the stream ran dry. Patches above barriers began in the catchment polygon above the reservoir and continued until the stream ran dry or another barrier was reached. Patch area (in hectares) and stream length (in kilometres) were delineated with the USGS NHDPlus GIS layer. We multiplied patch area by stream length to obtain the metric "patch size".

Complete surveys were conducted for eight brook trout habitat patches located in Rockingham County, Virginia, USA (Table 1; Fig. 1). The eight habitat patches occur within three subwatersheds (Dry River, Briery Branch, and Little River) within the North River Watershed (Fig. 1). The Dry River subwatershed contains patches DV-a, DV-b, and DN-a (-a denotes above a dam, -b denotes below a dam). The Briery Branch subwatershed contains the patches BB-a and BB-b. The Little River subwatershed contains patches LR-a, LR-b, and SF-a. The Skidmore Fork (SF) flows into the Little River and lies within the same subwatershed. LR-b is the closest downstream patch to SF-a that contains brook trout (Fig. 1). No brook trout were captured directly downstream of SF-a, despite sampling effort similar to other sites. The mean date of construction for the five dams that separate the five above-barrier patches examined is 1966 (Table 1).

The sampling protocol consisted of single-pass electrofishing surveys of entire habitat patches during July–August 2010 and July–August 2011. Sampling during late summer allowed age-0 brook trout to become large enough to be captured efficiently while still enabling year-class differentiation based upon length (Hudy et al. 2000). We constructed a length–frequency histogram for each sample to differentiate young of the year (YOY) from

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**Fig. 1.** Map of north-central Virginia, USA, showing the eight brook trout habitat patches within the portion of the North River Watershed examined in this study. DV, Dry River; DN, Dry Run; BB, Briery Branch; LR, Little River; SF, Skidmore Fork. Above-dam sites are denoted by -a, below-dam sites are denoted by -b. The wider boundary represents the hypothesized historical range of brook trout in this river system.
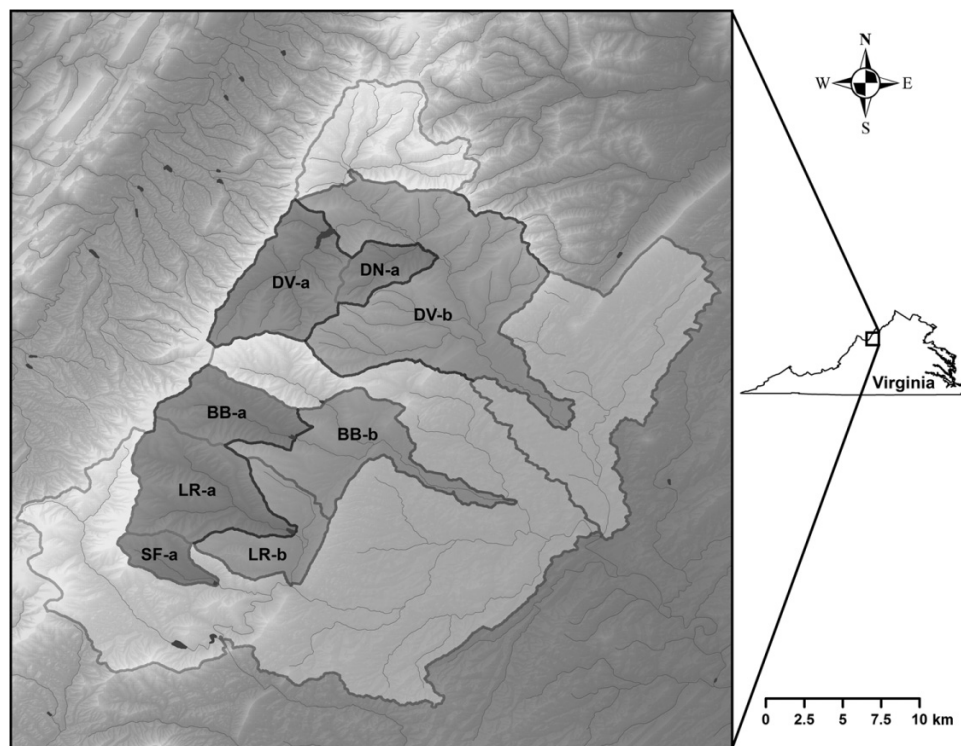


over-yearlings (adults; see supplementary material Fig. S1[1]). Length–frequency histograms were strongly bimodal (Fig. S1[1]). The mean (SD) cutoff length we used to define YOY for all patches was 93.5 mm (9.9). Habitat area was also reduced in late summer, which allowed entire above-barrier and a substantial portion of below-barrier patches to be surveyed. We conducted mark–recapture population estimates for the entire habitat patch on both YOY and adults. Upon capture, individual length (nearest mm, total length (TL)) and location (nearest upstream metre) were recorded, and a tissue sample (anal fin clip) was taken as a source of genetic material and to serve as a mark for mark–recapture purposes. Patches were resampled within 2 weeks of the initial capture event to estimate the proportion of marked to unmarked fish and abundance was estimated with the Lincoln–Petersen estimator (Otis et al. 1978). We estimated abundances separately for YOY and adults.

### Genotyping

All populations were genotyped at eight microsatellite loci (*SfoC113*, *SfoD75*, *SfoC88*, *SfoD100*, *SfoC115*, *SfoC129*, *SfoC24*; King et al. 2012) and *SsaD237* (King et al. 2005) following protocols for DNA extraction and amplification detailed in King et al. (2005). Loci were electrophoresed on an ABI Prism 3130xl genetic analyzer (Applied Biosystems Inc., Foster City, California), and alleles were hand-scored using GENEMAPPER version 3.2 and PEAK SCANNER version 1.0 software (Applied Biosystems Inc.). All data are available from the authors upon request.

### Genetic data analysis

We used GENEPOP version 4.0.10 (Rousset 2008) to test for deviations from Hardy–Weinberg (HW) expectations and linkage disequilibrium (LD). We used a sequential Bonferroni correction

at an alpha ($\alpha$) of 0.05 to correct for inflated type I error rates due to multiple testing (Rice 1989). We used GENODIVE version 2.0b22 (Meirmans and Van Tienderen 2004) to estimate allele frequencies, observed ($H_O$) and expected ($H_E$) heterozygosity per locus and population, mean number of alleles ($A$), and $F_{IS}$. We used FSTAT version 2.9.3.2 (Goudet 2001) to estimate mean allelic richness per population (AR; mean number of alleles scaled to the smallest sample size).

Family structure within single-cohort samples can cause deviations from HW expectations, elevated LD, and bias analyses of genetic structure (Allendorf and Phelps 1981; Anderson and Dunham 2008; Rodriguez-Ramilo and Wang 2012). To minimize any biases associated with family structure, we first reconstructed full-sibling families within each sample with COLONY version 1.2 (Wang 2004). It is worth noting that previous simulation-based analyses for three of the cohort samples examined here revealed mean sibship reconstruction accuracies of 91.2% (range 87.4%–93.2%; Whiteley et al. 2012). Second, we randomly selected one individual per family from each cohort sample to obtain a random subset of the data that should be free of family structure effects (Rodriguez-Ramilo and Wang 2012). We did not resample the data to form multiple random subsets because we were removing full-siblings that are by definition highly genetically similar, and therefore resampled subsets would be assured of producing similar results. We performed analyses with the entire data set and with the randomly chosen subset of the data. To quantify aspects of the distribution of full-sibling families within each site, we calculated family evenness (FE) for each cohort sample according to the following equation:

$$FE = \frac{H'}{H'_{Max}}$$

where $H' = -\sum_1^S p_i \ln(p_i)$ and $H'_{Max} = \ln(S)$ (Mulder et al. 2004). $S$, which usually represents the number of species in an evenness calculation, here represented the number of families, and $p_i$ represented the proportion of the $i$th family.

We constructed a generalized linear model (GLM) to relate counts of either significant violations of HW proportions or significant tests of LD per population (separate response variables) to variation in family structure (number of full-sibling families and evenness of full-sibling family distributions), sample size, and AR (predictor variables). We used GLMs with a Poisson error structure and a log link function and performed analyses with R version 2.15.0 (R Development Core Team 2006) and the lme4 package. We used a logit transformation ($\ln[p/(1 − p)]$) for the FE proportion (Warton and Hui 2011). We included sample size ($N$) and AR in these models as predictors because they are likely to influence power to detect significant LD.

To test for further population-level genetic structure within patches, beyond family-level structure revealed by sibship reconstruction, we used STRUCTURE version 2.3.1 (Pritchard et al. 2000). We used the subset of the data that contained only one randomly selected individual per full-sibling family for each cohort within each patch. We used 100 000 replicates and 20 000 burn-in cycles under an admixture model. We inferred a separate Dirichlet parameter for degree of admixture ($\alpha$) for each population. We used the correlated allele frequencies model with an initial $\lambda$ of 1, where $\lambda$ parameterizes the allele frequency prior and is based on the Dirichlet distribution of allele frequencies. We allowed $F$ to assume a different value for each population, which allows for different rates of drift among populations. We performed five runs for each of $K = 1$ to 4.

We used Meirmans and Hedrick's unbiased estimator $G''_{ST}$ (Meirmans and Hedrick 2011) for estimates of overall and pairwise $F'_{ST}$. We used Nei's unbiased estimator of $G_{ST}$ (Nei 1987) for estimates of overall and pairwise $F_{ST}$. Both $F'_{ST}$ and $F_{ST}$ were calculated with GENODIVE. We combined locus-specific exact tests for allele frequency (genic) differentiation implemented in GENEPOP with Fisher's method. This test assumes that under the null hypothesis of no allele frequency differentiation at any of the eight loci, the quantity $-2\sum\ln P_j$ is distributed as $x^2$ with df $= 2k$, where $k$ is the number of loci and $P_j$ is the $P$ value for the $j$th locus (Ryman et al. 2006). We used the less conservative B-Y FDR (Benjamini and Yekutieli 2001) correction method to control the type I error rate ($\alpha = 0.05$) for results from this combined test following Narum (2006). To further examine the partitioning of genetic variation between successive cohorts within patches, we performed a standardized AMOVA that included each patch for which we had successive samples as a separate group. We estimated standardized $\varphi'_{SC}$ (divergence between samples of successive cohorts within patches) and standardized $\varphi'_{CT}$ (divergence among patches) with GENODIVE. Principal components analysis (PCA) of sample allele frequencies was used to visualize patterns of population differentiation. We performed eigenanalysis of the covariance matrix with GENODIVE.

We also estimated the effective number of breeders ($N_b$) for each cohort. When applied to single-cohort samples, single-sample $N_e$ estimators provide an estimate of the effective number of breeders that gave rise to that cohort (Waples and Do 2010). All $N_b$ estimates were generated using the single-sample linkage disequilibrium method within the program LDNe version 1.31 (Waples and Do 2008). A monogamous mating model was assumed based on a report that 80% of parents contributed to only a single family in two headwater stream brook trout populations (Coombs 2010). $N_b$ estimates were derived using a minimum allele frequency cutoff ($P_{crit}$) of 0.02, which has been shown to provide

an adequate balance between precision and bias across sample sizes (Waples and Do 2008). 95% confidence intervals were generated using the jackknife approach.

### Statistical analyses

We used a linear model to examine the effect of patch size on estimates of genetic variation within patches and $N_b$. We also tested for patch size-dependent variation in components of family structure as a possible explanation for patch size-dependent variation in $N_b$. For patch size, we combined patch area and stream length (patch area × stream length × $10^{-3}$) to form one predictor variable that we then log-transformed. Response variables in separate models included mean observed number of alleles ($A_O$), mean AR, mean expected heterozygosity ($H_S$), point estimates of $N_b$, mean full-sibling family size (FS), or FE. We used a logit transformation for $H_S$ and FE (Warton and Hui 2011). We examined above-barrier patches separately from below-barrier patches and performed separate analyses for each cohort (2010 or 2011). One-tailed significance values were used for $A_O$, AR, $H_S$, and $N_b$ because predictions were directional. All analyses were performed with the stats package in R.

## Results

### Genetic variation within patches

We examined variation at eight microsatellite loci in 2229 brook trout from eight habitat patches (Fig. 1). Mean patch size was 3315 ha (range 993–10 880 ha; Table 1). Mean stream length within patches was 14.4 km (range 5.1–40.0 km; Table 1). The mean of our combined patch size metric was 80.3 (range 5.1–435.2; Table 1). The mean estimate of census population size ($N_c$) for YOY was 406.5 (range 22–1285; Table 1). The mean estimate of $N_c$ for adults was 462.8 (range 43–1982; Table 1). For the entire data set, the mean $A_O$ per population ranged from 2.8 to 11.6, mean AR (standardized to $N = 27$) ranged from 2.8 to 8.8, and mean $H_S$ ranged from 0.392 to 0.797 (Table 2). The mean estimated number of full-sibling families was 48.9 (range 11–137), and the range of mean FS was 1.6–4.6 (Table 2). Mean FE was 0.911 (range 0.834–0.970). Point estimates of $N_b$ ranged from 4.9 to 191.2 (Table 2). The $N_b$ confidence interval for DN-a-2011 included infinity (Table 2). This result was due to small sample size ($N = 27$), despite exhaustive sampling and low genetic diversity. We deemed this estimate of $N_b$ to be unreliable and excluded it in subsequent analyses. We used estimates of $N_b$ based on 2011 YOY samples and estimates of $N_c$ based on 2010 adults to obtain $N_b/N_c$ ratios for four samples (Table 2). $N_b/N_c$ ratios ranged from 0.038 to 0.089 (Table 2).

The subset of the data that contained only one randomly selected individual per full-sibling family contained a total of $N = 685$ individuals. The estimated number of families per patch became each site's sample size (Table 2). This subset of the data yielded similar estimates of genetic variation within sites (Table 2). $A_O$ ranged from 2.6 to 10.9, AR (standardized to $N = 11$) ranged from 2.6 to 6.9, and $H_S$ ranged from 0.409 to 0.794 (Table 2).

For the entire data set, we performed 110 tests for departures from HW proportions. Prior to correction for multiple tests, 57 (52%) of these tests were significant ($P < 0.05$), where six were expected by chance ($\alpha = 0.05$). Following sequential Bonferroni correction for 110 tests ($\alpha = 0.05$; initial nominal $P$ value = 0.00045), 31 tests remained significant (Table 2, Table S1[1]). Random selection of one individual per family largely removed the signal of HW departures. For this randomly chosen subset of the data, nine of 110 (8%) tests for HW violations were significant prior to correction for multiple tests ($P < 0.05$), where six were expected by chance with an $\alpha$ of 0.05. There were no apparent patterns across populations or loci for these significant departures from HW proportions. Following sequential Bonferroni correction for 110 tests ($\alpha = 0.05$), two tests remained significant (SfoD237 in BB-a-2011 and SfoC88 in SF-a-2010).

**Table 2.** Genetic summary statistics for young-of-the-year (YOY) brook trout captured in eight habitat patches in Virginia, USA.

| Site name | Site code | Sample year | HW | LD | $A_O$ | $A_O$-RS | AR | AR-RS | $H_S$ | $H_S$-RS | Families | Mean FS | FE | $\hat{N}_b$ | $\dfrac{\hat{N}_b}{\hat{N}_c}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dry River-a | DV-a | 2010 | 6 | 25 | 10.9 | 10.1 | 8.2 | 6.9 | 0.780 | 0.792 | 101 | 3.8 | 0.897 | 66.6 (57.8–76.5) | — |
| Dry River-a | DV-a | 2011 | 7 | 26 | 11.6 | 10.9 | 8.8 | 6.9 | 0.797 | 0.794 | 137 | 3.7 | 0.901 | 75.0 (60.9–91.4) | 0.038 |
| Dry River-b | DV-b | 2010 | 0 | 1 | 8.8 | 8.5 | 7.8 | 6.5 | 0.777 | 0.778 | 57 | 1.7 | 0.949 | 191.2 (140.3–279.8) | — |
| Dry River-b | DV-b | 2011 | 0 | 2 | 9.3 | 8.9 | 7.9 | 6.4 | 0.770 | 0.780 | 41 | 1.6 | 0.970 | 152.8 (111.5–227.2) | — |
| Dry Run-a | DN-a | 2010 | 1 | 12 | 3.4 | 3.4 | 3.4 | 3.3 | 0.565 | 0.571 | 15 | 3.1 | 0.925 | 4.9 (3.8–8.7) | — |
| Dry Run-a | DN-a | 2011 | 0 | 0 | 2.8 | 2.6 | 2.8 | 2.6 | 0.392 | 0.409 | 13 | 2.1 | 0.941 | 40.2 (12.6–∞) | — |
| Briery Branch-a | BB-a | 2010 | 0 | 14 | 7.1 | 6.8 | 6.4 | 5.7 | 0.728 | 0.719 | 25 | 2.9 | 0.866 | 26.2 (20.7–33.0) | — |
| Briery Branch-a | BB-a | 2011 | 5 | 15 | 7.6 | 7.3 | 6.4 | 5.9 | 0.702 | 0.740 | 30 | 3.0 | 0.834 | 32.6 (26.1–40.6) | 0.089 |
| Briery Branch-b | BB-b | 2011 | 1 | 7 | 8.1 | 8.1 | 7.0 | 6.2 | 0.721 | 0.729 | 38 | 2.6 | 0.946 | 59.8 (50.4–71.3) | — |
| Little River-a | LR-a | 2010 | 4 | 23 | 9.5 | 8.8 | 6.8 | 5.8 | 0.712 | 0.709 | 87 | 3.4 | 0.883 | 46.0 (39.6–53.2) | — |
| Little River-a | LR-a | 2011 | 6 | 23 | 8.8 | 8.4 | 6.8 | 5.8 | 0.717 | 0.713 | 90 | 4.2 | 0.923 | 53.9 (44.0–65.2) | 0.074 |
| Little River-b | LR-b | 2011 | 0 | 3 | 8.0 | 7.1 | 6.8 | 5.5 | 0.645 | 0.669 | 26 | 2.2 | 0.938 | 70.3 (53.0–97.1) | — |
| Skidmore Fork-a | SF-a | 2010 | 1 | 5 | 5.4 | 4.9 | 4.8 | 4.9 | 0.543 | 0.640 | 11 | 4.6 | 0.864 | 10.1 (5.2–15.1) | — |
| Skidmore Fork-a | SF-a | 2011 | 0 | 6 | 4.1 | 3.8 | 3.9 | 3.7 | 0.520 | 0.571 | 14 | 3.6 | 0.915 | 17.1 (10.5–26.5) | 0.064 |

**Note:** Measures are as follows: HW, number of significant departures from Hardy–Weinberg proportions following sequential Bonferroni correction ($\alpha = 0.05$) for 110 tests; LD, number of significant tests for linkage disequilibrium following sequential Bonferroni correction ($\alpha = 0.05$) for 385 pairwise tests; $A_O$, mean number of observed alleles for the entire data set and for the random subsample (RS) of one full-sibling per family; AR, allelic richness standardized to $N = 27$ and $N = 11$ for the random subsample (AR-RS); $H_S$, mean expected heterozygosity for the entire data set and random subsample ($H_S$-RS); Families, number of estimated full-sibling families; mean FS, mean number of individuals per full-sibling family; FE, family evenness; $\hat{N}_b$, LDNe-based single-sample estimates of the effective number of breeders that gave rise to that year's YOY. The $\hat{N}_b/\hat{N}_c$ ratio is based on $\hat{N}_b$ of the 2011 YOY and $\hat{N}_c$ (estimated census size) of 2010 adults.

We constructed a model to further examine the widespread signal of deviation from HW proportions. We predicted that family structure within the cohort-specific samples would be the most likely cause of the large amount of significant HW deviations, along with factors that influence power ($N$ and AR). In a GLM that included number of significant HW proportion tests per population as the response variable and FE, number of families, $N$, and AR as explanatory factors, FE (logit-transformed; $z = -2.1$, $P = 0.03$) had the largest relative effect. Sample size ($z = 1.8$, $P = 0.07$), number of full-sibling families ($z = -1.2$, $P = 0.24$), and AR ($z = 0.26$, $P = 0.80$) had smaller and nonsignificant relative effects. Combined, these four predictors explained a substantial proportion of variation in the number of significant violations of HW proportions per population (explained deviance = 77.0%).

Prior to correction for multiple tests, 256 of 385 (67%) tests for LD were significant ($P < 0.05$) for the entire data set. Following sequential Bonferroni correction for 385 tests ($\alpha = 0.05$; initial nominal $P$ value = 0.00013), 162 tests remained significant, and the mean number of significant LD tests per population was 11.6 (range 0–26; Table 2). Random selection of one individual per family largely removed the signal of LD. For this subset of the data, 29 of 379 (8%) tests for LD were significant without correction for multiple tests ($P < 0.05$), where 19 were expected by chance with an $\alpha$ of 0.05. Two of these tests remained significant following sequential Bonferroni correction for 379 tests ($\alpha = 0.05$). In a GLM that included number of significant LD tests per population as the response variable and FE, number of families, $N$, and AR as explanatory factors, FE (logit-transformed; $z = -3.8$, $P = 0.0001$) had the largest relative effect followed by sample size ($z = 2.1$, $P = 0.04$). Number of full-sibling families ($z = -1.0$, $P = 0.31$) and AR ($z = 0.06$, $P = 0.96$) had small and nonsignificant relative effects. Combined, these four predictors explained a substantial proportion of variation in the number of LD tests per population (explained deviance = 76.8%).

Following the random selection of one individual per family, there was no evidence of any further population-level structure within any of the patches. $K = 1$ had the greatest support for within-patch STRUCTURE models in all cases.

## Genetic differentiation among patches

There was evidence for strong overall genetic differentiation among patches. To avoid possible biases associated with family structure in analyses of genetic differentiation among patches, we performed analyses with the randomly selected (one individual

per full-sibling family) subset of the data. Eighty-eight of 91 (97%) combined pairwise tests for genic differentiation were significant based on Fisher's method and following B-Y FDR correction (nominal $P = 0.0098$; Table 3). Overall $F'_{ST}$ was 0.397 (95% CI: 0.322–0.525). Overall $F_{ST}$ was 0.124 (95% CI: 0.096–0.159). Pairwise $F'_{ST}$ values ranged from −0.014 to 0.846 (Table 3). Pairwise $F_{ST}$ ranged from −0.004 to 0.431.

The smallest patch (DN-a) exhibited the most between-cohort allele frequency divergence; otherwise, allele frequencies were relatively stable between successive cohorts (Table 3). Samples from successive cohorts from the same patch tended to have the lowest genetic differentiation (Table 3). The mean (SD) $F'_{ST}$ for successive cohorts was 0.055 (0.123), mean $F_{ST}$ was 0.027 (0.063), and the mean number of loci with significant exact tests for genic differentiation following B-Y FDR correction was 1.5 (2.1; Table 3). Successive cohorts in DN (2010 and 2011) were substantially more genetically differentiated than the other successive cohort pairwise comparisons ($F'_{ST} = 0.304$; Table 3). With this population excluded, mean (SD) $F'_{ST}$ for successive cohorts was 0.005 (0.12), mean $F_{ST}$ was 0.001 (0.003), and the mean number of loci with significant exact tests for genic differentiation following B-Y FDR correction was 0.8 (1.3). To further examine the partitioning of genetic variation between successive cohorts within patches, we performed a standardized AMOVA that included each patch for which we had successive samples as a separate group. Standardized $\varphi'_{SC}$ (divergence between samples within patches) was 0.028. Standardized $\varphi'_{CT}$ (divergence among patches) for this subset of the data was an order of magnitude higher, 0.297.

The smallest patches also exhibited the greatest genetic divergence from other sites (Fig. 2). PC1 explained 43% of the variance in the population allele frequencies. The two sites with the smallest $\hat{N}_b$, DN-a and SF-a, were the most genetically divergent sites along PC1, followed by LR-a and LR-b. The remainder of the sites had similar PC1 scores. PC2 explained 19% of the variance. DN-a and SF-a were also highly divergent along PC2. LR-a was divergent from LR-b along PC2. DV-a (2010 and 2011), DV-b (2010 and 2011), and BB-b were all highly similar for both PC1 and PC2. BB-a (2010 and 2011) was only slightly divergent from these sites along PCs 1 and 2. PC3 explained 13% of the variance. This axis revealed divergence of the three BB sites from others (except BB-b-2011 from DN-a-2011) as well as divergence above and below the dam in BB. LR-a-2010 was divergent from LR-a-2011 and from LR-b along

**Table 3.** Genetic differentiation among 14 cohort samples of brook trout YOY from eight habitat patches in Virginia, USA.

| Site | DV-a-2010 | DV-a-2011 | DV-b-2010 | DV-b-2011 | DN-a-2010 | DN-a-2011 | BB-a-2010 | BB-a-2011 | BB-b-2011 | LR-a-2010 | LR-a-2011 | LR-b-2010 | SF-a-2010 | SF-a-2011 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DV-a-2010 | — | 3 | 6 | 5 | 8 | 8 | 7 | 7 | 8 | 8 | 7 | 6 | 7 | 8 |
| DV-a-2011 | 0.011 | — | 7 | 6 | 8 | 8 | 7 | 7 | 8 | 8 | 8 | 6 | 8 | 8 |
| DV-b-2010 | 0.095 | 0.084 | — | 0 | 8 | 8 | 7 | 7 | 5 | 8 | 8 | 7 | 7 | 7 |
| DV-b-2011 | 0.065 | 0.066 | **0.005** | — | 8 | 7 | 7 | 7 | 6 | 8 | 7 | 7 | 5 | 7 |
| DN-a-2010 | 0.484 | 0.449 | 0.462 | 0.438 | — | 5 | 7 | 8 | 7 | 8 | 8 | 8 | 7 | 7 |
| DN-a-2011 | 0.620 | 0.618 | 0.570 | 0.570 | 0.304 | — | 8 | 8 | 8 | 8 | 8 | 8 | 7 | 8 |
| BB-a-2010 | 0.312 | 0.293 | 0.355 | 0.282 | 0.467 | 0.695 | — | 0 | 5 | 7 | 7 | 7 | 8 | 8 |
| BB-a-2011 | 0.300 | 0.271 | 0.337 | 0.267 | 0.444 | 0.684 | **−0.014** | — | 5 | 7 | 7 | 7 | 7 | 8 |
| BB-b-2011 | 0.253 | 0.227 | 0.263 | 0.199 | 0.377 | 0.625 | 0.083 | 0.089 | — | 7 | 7 | 4 | 7 | 7 |
| LR-a-2010 | 0.262 | 0.25 | 0.279 | 0.246 | 0.592 | 0.801 | 0.331 | 0.309 | 0.221 | — | 1 | 6 | 6 | 7 |
| LR-a-2011 | 0.265 | 0.247 | 0.286 | 0.243 | 0.597 | 0.790 | 0.275 | 0.262 | 0.195 | 0.017 | — | 6 | 7 | 7 |
| LR-b-2010 | 0.248 | 0.245 | 0.277 | 0.219 | 0.541 | 0.741 | 0.283 | 0.252 | 0.174 | 0.209 | 0.169 | — | 6 | 6 |
| SF-a-2010 | 0.377 | 0.389 | 0.418 | 0.341 | 0.632 | 0.801 | 0.371 | 0.397 | 0.359 | 0.396 | 0.401 | 0.358 | — | **0** |
| SF-a-2011 | 0.445 | 0.464 | 0.496 | 0.447 | 0.647 | 0.846 | 0.454 | 0.471 | 0.408 | 0.417 | 0.423 | 0.347 | **0.004** | — |

**Note:** $F'_{ST}$ is below the diagonal. Number of significant exact tests for genic differentiation is above the diagonal following B-Y FDR correction for multiple tests (nominal $P = 0.0098$). Bold values were not significant following Fisher's method for combining $P$ values across the eight exact tests per cohort sample (B-Y FDR correction, nominal $P = 0.0098$).

**Table 4.** Summary of genetic diversity in above-barrier relative to adjacent below-barrier habitat patches.

| River basin | Above site | Below Site | Patch size (above) | $A_O$ (a/b) | Percentage change $A_O$ | AR (a/b) | Percentage change AR | $H_S$ (a/b) | Percentage change $H_S$ | $N_b$ (a/b) | Percentage change $N_b$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Dry River | DV-a-2010 | DV-b-2010 | 104.3 | 10.1/8.5 | 19 | 6.9/6.5 | 6 | 0.792/0.778 | 2 | 66.6/191.2 | −65 |
| Dry River | DV-a-2011 | DV-b-2011 | 104.3 | 10.9/8.9 | 22 | 6.9/6.4 | 8 | 0.794/0.780 | 2 | 75.0/152.8 | −51 |
| Dry Run | DN-a-2010 | DV-b-2010 | 9.9 | 3.4/8.5 | −60 | 3.3/6.5 | −49 | 0.571/0.778 | −27 | 4.9/191.2 | −97 |
| Dry Run | DN-a-2011 | DV-b-2011 | 9.9 | 2.6/8.9 | −71 | 2.6/6.4 | −59 | 0.409/0.780 | −48 | — | — |
| Briery Branch | BB-a-2011 | BB-b-2011 | 14.9 | 7.3/8.1 | −10 | 5.9/6.2 | −5 | 0.740/0.729 | 2 | 32.6/59.8 | −45 |
| Little River | LR-a-2011 | LR-b-2011 | 52.3 | 8.4/7.1 | 18 | 5.8/5.5 | 5 | 0.713/0.669 | 7 | 53.9/70.3 | −23 |
| Little River | SF-a-2011 | SF-b-2011 | 5.1 | 3.8/7.1 | −46 | 3.7/5.5 | −33 | 0.571/0.669 | −15 | 17.1/70.3 | −76 |

**Note:** Comparisons were made for adjacent above-below pairs in years where both samples were available. Patch size was calculated as patch area × stream length × $10^{-3}$, and only above-barrier patch size is shown here. DN-a was compared with DV-b, and SF-a was compared with LR-b. Mean number of alleles ($A_O$), mean AR, $H_S$, and $N_b$ are shown for above- and adjacent below-barrier patches (a/b). Percentage change (above relative to below) is negative when values were lower in above-barrier patches than in below-barrier patches and positive if above-barrier patches had greater values than below-barrier patches.

PC3. All the cohort comparisons for sites DV-b and DV-a were also similar along PC3 (Fig. 2).

Genetic drift appears to be the predominant evolutionary process responsible for the genetic differentiation observed. The relationship between within-stream (above relative to below barrier) genetic differentiation, and estimates of within-population genetic diversity of the above-barrier population in each pair was strongly negative. We included pairs of cohorts sampled above and below barriers in the same year for a total of seven comparisons. The correlation between above–below pairwise $F'_{ST}$ and above-barrier population $A_O$ was −0.99 ($t = −6.91$, $P = 0.0005$), above-barrier population AR was −0.98 ($t = −11.42$, $P < 0.0001$), and above-barrier population $H_S$ was −0.96 ($t = −7.89$, $P = 0.0003$).

Analyses based on the entire data set yielded slightly greater estimates of genetic differentiation. For example, overall $F'_{ST}$ was 0.434 (95% CI: 0.357–0.558). Standardized $\varphi'_{SC}$ was 0.061, and standardized $\varphi'_{CT}$ was 0.328.

### Effect of barriers and patch size on genetic variation and $N_b$

Maintenance of genetic diversity in above-barrier patches was strongly patch size-dependent. The two largest above-barrier patches (DV-a and LR-a) possessed more genetic diversity than adjacent below-barrier patches (Table 4, Fig. 3). These two largest above-barrier patches had an average of 20% greater $A_O$, 7% greater AR, and 4% greater $H_S$ than adjacent below-barrier patches (Table 4). Relatively small above-barrier patches (DN-a, SF-a, and BB-a) had much lower genetic diversity than adjacent below-barrier patches (Table 4, Fig. 3). The three smallest above-barrier patches had an average of 47% lower $A_O$, 37% lower AR, and 22% lower $H_S$ than adjacent below-barrier patches. There was a strong

positive effect of patch size on genetic diversity ($A_O$, AR, and $H_S$). This relationship was significant ($P < 0.05$) in above-barrier patches for $A_O$ in 2010 and 2011, AR in 2011, and $H_S$ in 2010 (Table 5). This relationship was significant for below-barrier patches for $H_S$ in 2011, despite inclusion of only three patches in the analysis.

$N_b$ was lower in above-barrier patches than in adjacent below-barrier patches in all cases (Table 4; Fig. 3). The two largest patches had an average of 46% lower $N_b$ than adjacent below-barrier patches (Table 4). The three smallest patches had an average of 73% lower $N_b$ than adjacent above-barrier patches (Table 4). $N_b$ was extremely reduced in the two smallest above-barrier patches (97% in DN-a and 76% in SF-a; Table 4). The relationship between $N_b$ and patch size was positive and significant in both 2010 and 2011 for above-barrier patches but not for the three below-barrier patches (Table 5). This pattern did not appear to be driven by patch size-dependent variation in reproductive success, as measured by mean FS or FE. The relationship between mean FS or FE and patch size was not significant in any of the comparisons (Table 5).

### Discussion

Our analysis revealed that patch size mediated the effect of isolation by dams on within-population (patch) genetic variation. For the patches that occur above dams, we observed a strong positive relationship between patch size and genetic variation. That is, the largest above-barrier patches have maintained substantial genetic variation while the smallest isolated patches showed dramatically reduced levels. This is consistent with previous studies, for example, a similar strong relationship among patch size and genetic variation and $N_e$ was observed in long-isolated (~10 000 years) coastal cutthroat trout (*Oncorhynchus*

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**Fig. 2.** Principal components analysis of allele frequency variation for brook trout from eight Virginia habitat patches. Black circles represent above-barrier populations, and grey triangles represent below-barrier populations. Proportion of variation attributable to PC axes 1 and 2 (panel *a*) and PC axes 1 and 3 (panel *b*) is shown. Population (patch) labels are shown for all sites.
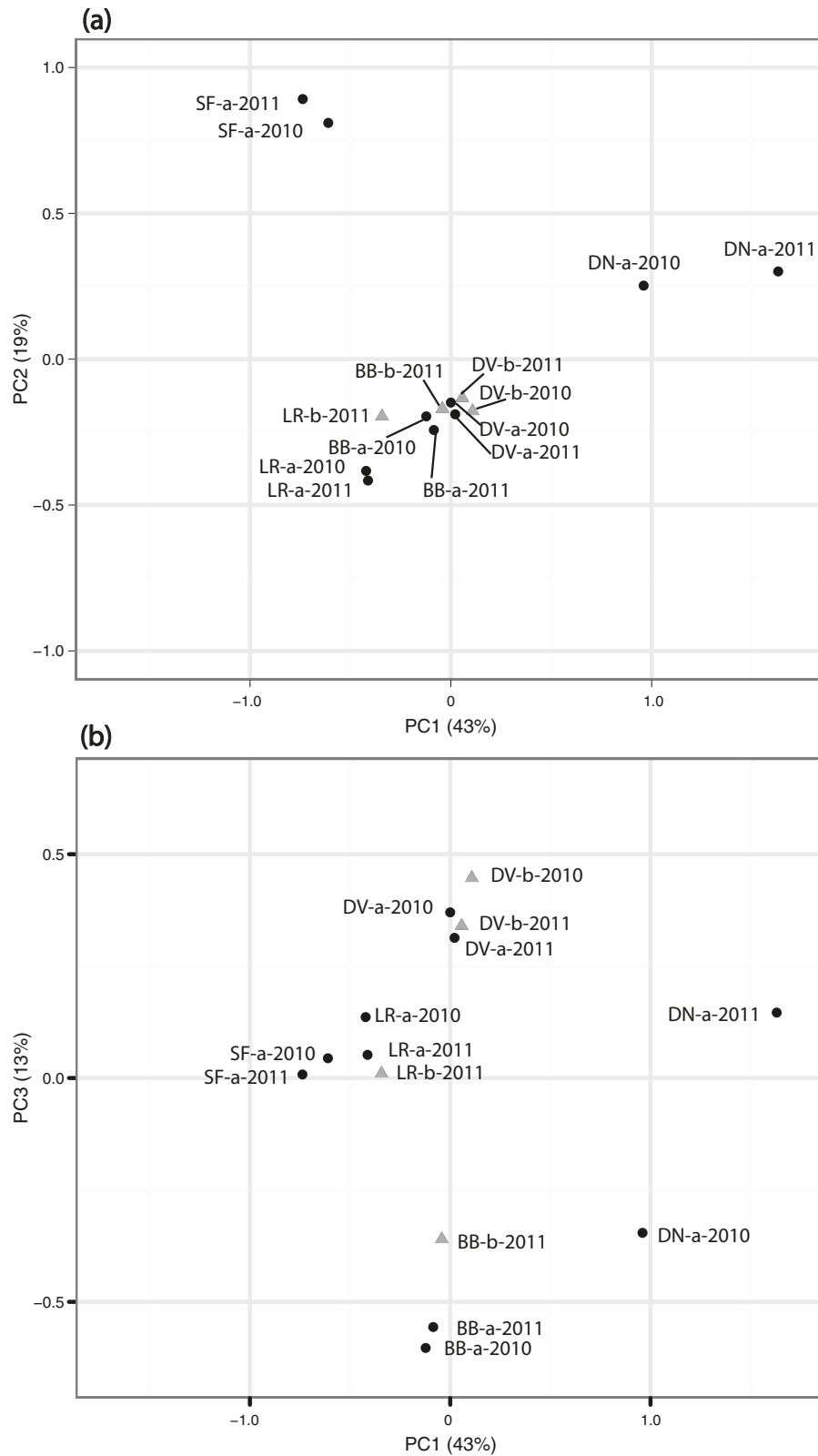
**Fig. 3.** Genetic summary statistics ($\hat{A}_O$, $\widehat{AR}$, and $\hat{H}_S$) and estimates of $\hat{N}_b$ for brook trout in the eight habitat patches examined. Patches are shown within each of three subwatersheds (Dry River, Briery Branch, and Little River) of the North River Watershed. Cohorts sampled above barriers are shown as black circles and below barriers as grey triangles. Symbol size is proportional to the log of patch size. Patch abbreviations are shown to the right of symbols; labels occur at the approximate midpoint between cohort-specific estimates within a patch.
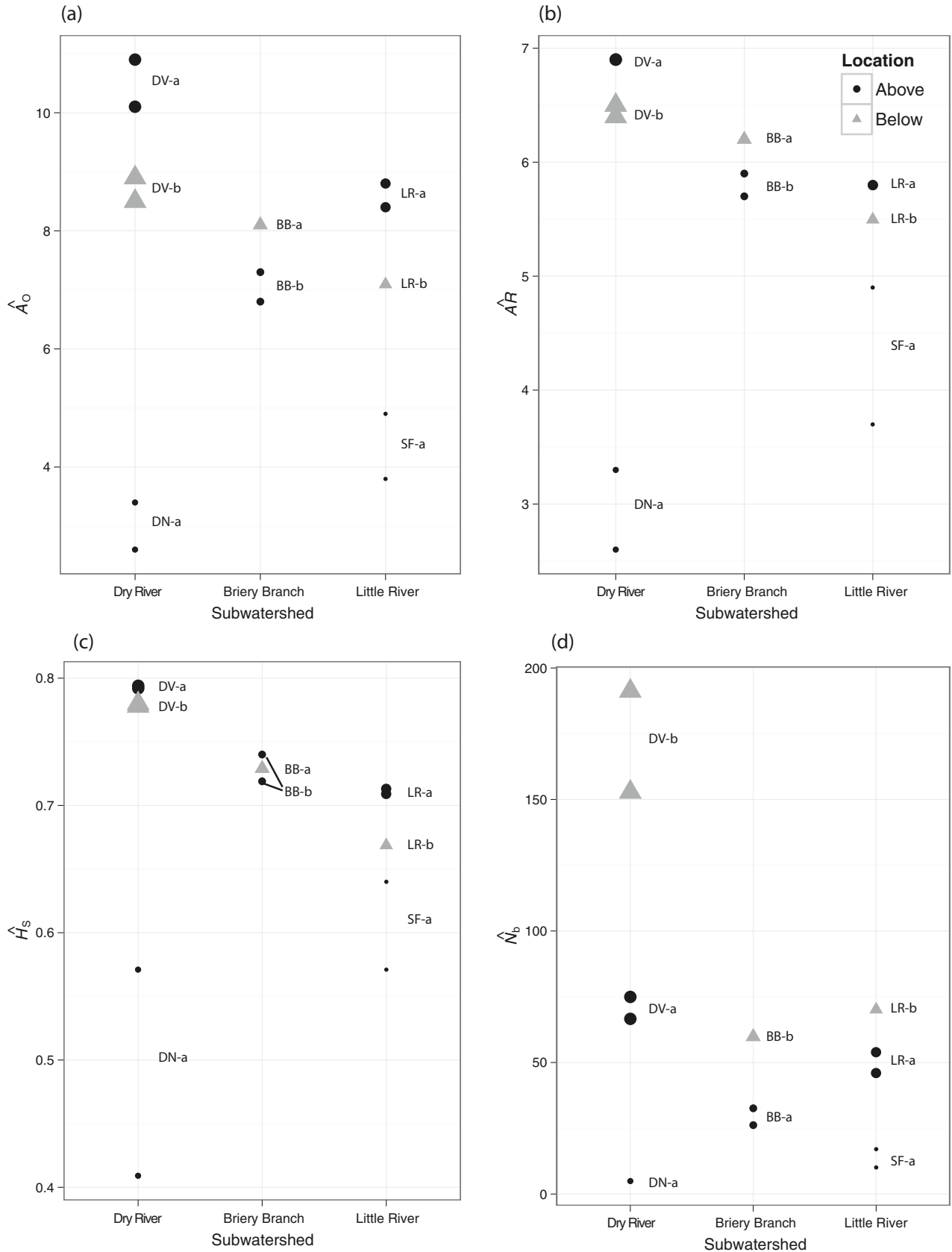
**Table 5.** Linear models of mean number of alleles ($A_O$), allelic richness (AR), mean expected heterozygosity ($H_s$), effective number of breeders ($N_b$), mean full-sibling family size (Mean FS), and family evenness (FE) as response variables and patch size (log-transformed) as the predictor variable.

| Location | Year | N | t | P | $R^2$ |
|---|---|---|---|---|---|
| $A_O$ | | | | | |
| Above | 2010 | 5 | 3.89 | 0.015 | 0.84 |
| Above | 2011 | 5 | 3.84 | 0.016 | 0.83 |
| Below | 2011 | 3 | 4.67 | 0.067 | 0.96 |
| AR | | | | | |
| Above | 2010 | 5 | 2.0 | 0.072 | 0.57 |
| Above | 2011 | 5 | 2.4 | 0.047 | 0.66 |
| Below | 2011 | 3 | 2.0 | 0.146 | 0.81 |
| $H_S$ | | | | | |
| Above | 2010 | 5 | 2.5 | 0.046 | 0.67 |
| Above | 2011 | 5 | 1.9 | 0.077 | 0.55 |
| Below | 2011 | 3 | 6.3 | 0.050 | 0.98 |
| $N_b$ | | | | | |
| Above | 2010 | 5 | 6.0 | 0.005 | 0.92 |
| Above | 2011 | 4 | 9.2 | 0.006 | 0.98 |
| Below | 2011 | 3 | 1.9 | 0.153 | 0.79 |
| **Mean FS** | | | | | |
| Above | 2010 | 5 | −0.3 | 0.764 | 0.04 |
| Above | 2011 | 5 | 1.1 | 0.356 | 0.28 |
| Below | 2011 | 3 | −1.0 | 0.508 | 0.49 |
| FE | | | | | |
| Above | 2010 | 5 | 0.2 | 0.849 | 0.01 |
| Above | 2011 | 5 | −0.2 | 0.875 | 0.01 |
| Below | 2011 | 3 | 4.8 | 0.130 | 0.96 |

**Note:** The 2010 and 2011 cohorts were examined separately for the above-barrier patches. Only 2011 below-barrier cohort samples were used. The estimate of $N_b$ for DN-a-2011 was excluded from the analysis (see text for details).

*clarkii clarkii*) populations (Whiteley et al. 2010). Studies of populations above relatively recent man-made barriers have shown a weak relationship between patch size and within-population genetic variation (e.g., Yamamoto et al. 2004). Here we demonstrate the strongest relationship between patch size and genetic variation shown to date for populations recently (~50 years) isolated by man-made barriers (dams). Our results suggest that a simple measure of habitat area (patch size), the combination of patch area and stream length, may be highly useful for the prediction of genetic diversity maintenance across a much larger number of eastern brook trout populations and perhaps those of other headwater fishes. However, it is possible that the strength of the effect of patch size was due to the similarity in the quality of habitat within the patches examined. All of these habitats face intermittent drying in the summer, with flashy and highly variable stream flow throughout the year. Differences in patch quality could confound the relationship between patch size and genetic variation if we had included a wider variety of patch types. That is, large patches with poor habitat quality might be predicted to have lower genetic variation than small patches with high habitat quality (Neville et al. 2006). An analysis of a wider range of patches that vary in habitat quality will be necessary to test whether patch size alone is a sufficient metric to predict levels of genetic diversity and $N_e$ for eastern brook trout populations.

Our study reveals that location below a barrier does not necessarily lead to greater maintenance of genetic diversity. Most studies of isolated populations of headwater fishes have found that above-barrier patches harbor populations with reduced genetic variation relative to adjacent below-barrier patches (Taylor et al. 2003; Yamamoto et al. 2004; Neville et al. 2006; Guy et al. 2008; Whiteley et al. 2010; Kitanishi et al. 2012). A surprising result from our study was that the two largest above-barrier patches had greater genetic variation than adjacent below-barrier patches. This suggests that below-barrier patches can lose genetic variation at a faster rate than large above-barrier patches. In addition, the positive relationship between patch size and genetic variation in the below-barrier patches, despite the inclusion of only three patches, suggests that connectivity is low or absent even without dams demarking lower patch boundaries. This has two important implications: (*i*) large above-barrier brook trout patches have been able to retain substantial genetic variation and should have relatively high persistence probabilities, and (*ii*) patches should not be assumed to be safe from extinction risk simply because they occur below a barrier. Below-barrier patches may be isolated because of high stream temperature, poor stream conditions, and (or) the presence of invasive species (Hudy et al. 2008). Other headwater fishes face similar downstream habitat limitations and stressors (Neville et al. 2006; Isaak et al. 2010), and therefore our results may be of general importance for headwater species.

The magnitude of $N_b$ estimates in above-barrier patches was strongly patch size-dependent. $N_b$ for organisms like the brook trout is likely to be most influenced by variance in individual reproductive success and the number of available spawning locations in a patch. Greater variance in reproductive success in smaller patches could be responsible for the observed relationship. However, the lack of relationship between average FS or FE with patch size in the above-barrier sites suggests that variance in reproductive success did not vary substantially among patches. The most likely explanation for strong positive patch size-dependent estimates of $N_b$ is that the amount of available spawning sites scales positively with patch size. More spawning sites in larger patches would allow more parents to contribute.

We also observed reduced $\hat{N}_b$ in all above-barrier patches relative to adjacent below-barrier patches. The primary effect of barriers may be to reduce available spawning habitat in above-barrier relative to below-barrier patches, even for larger above-barrier patches. Surprisingly, the two largest above-barrier patches had smaller $\hat{N}_b$ but greater estimates of genetic diversity than adjacent below-barrier patches. For these larger above-barrier patches, $N_b$ (and associated generational $N_e$) has apparently not been small enough to lead to a loss of genetic diversity at the time scale considered (approximately 50 years since dam construction), but loss of genetic diversity and elevated extinction risks could occur over longer time scales.

Our analysis demonstrates the utility of estimating $N_b$ from single cohorts for iteroparous species with overlapping generations and variable age at maturity. Estimates of $N_b$ were similar among years within sites. It might be possible to use future estimates of $N_b$ from these sites, obtained with the appropriate sampling strategy (Whiteley et al. 2012), to monitor population trend (Tallmon et al. 2010).

The significant negative relationship between genetic differentiation of the above-barrier populations and their within-population genetic diversity supports the hypothesis that drift has predominantly caused genetic differentiation of the above-barrier sites. The smallest above-barrier patches in this study exhibited the greatest genetic differentiation. There was little genetic differentiation of the largest above-barrier patches from adjacent below-barrier patches. Genetic drift may overwhelm natural selection in the smallest patches we examined and may prevent local adaptation. Inbreeding is also a substantial threat in the smallest patches. Alternatively, local adaptation should occur in the larger patches in this study, where the effects of genetic drift will be weaker.

A potential confounding factor on our inference regarding the effect of barriers on genetic differentiation is location of a patch in the landscape. Above-barrier patches occur in headwater locations. Gene flow to headwater reaches may be reduced by factors other than barriers. Furthermore, genetic differentiation of headwater from mainstem sites may have occurred prior to dam construction. It is likely that we would have observed greater genetic differentiation between large above-barrier and adjacent below-barrier patches had substantial divergence occurred prior to dam construction. Furthermore, lack of genetic differentiation between samples from the mainstem (DV-a-2010 and -2011) and an undammed headwater site within the DV-b patch (A. Whiteley, unpublished results) suggests that connectivity with the mainstem prevents genetic substructure at this scale. Headwater location alone does not appear to cause strong genetic differentiation in this study system. However, a more complete analysis of headwater sites that are not located above a dam would be necessary to further explore these possible confounding effects.

It is also important to note that we used single-cohort samples of YOY for this study. Family effects can cause bias in patterns of genetic structure (Allendorf and Phelps 1981; Rodriguez-Ramilo and Wang 2012). Family structure had a large effect on LD in our study. Further, genetic differentiation was slightly greater when we included all sampled individuals. Our use of a random subset of the data where we sampled one individual per family reduced the LD signal and provided generally lower estimates of genetic differentiation. This approach minimized any potential bias in reported patterns of genetic differentiation.

The smallest above-barrier patches in this study face the greatest threat of extirpation. These small patches exhibited markedly reduced genetic variation, very low $N_b$, and marked genetic differentiation from adjacent patches. The sizes of the two smallest patches in our study were 993 (SF) and 1217 (DN) hectares. The median patch size for over 2800 brook trout patches in the southeastern USA (855 ha) is smaller than our smallest patch (M. Hudy, unpublished data). These small and isolated patches that occur over a wide portion of the brook trout range may be highly jeopardized by continued loss of habitat, fragmentation, and climate change effects. More widespread genetic monitoring, based on an appropriate sampling design that allows precise and unbiased estimates of genetic variation and $N_b$ (Whiteley et al. 2012), will be necessary to determine the status of habitat patches throughout the brook trout range. Genetic rescue (Tallmon et al. 2004) may be an effective conservation approach to alleviate inbreeding depression and restore genetic diversity in the most threatened patches.

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